JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1998, p. 3497–3504 Vol. 36, No. 12
0095-1137/98/$04.00+0

Expanded Diversity among Californian *Borrelia* Isolates and Description of *Borrelia bissettii* sp. nov. (Formerly *Borrelia* Group DN127)

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Received 2 July 1998/Returned for modification 27 August 1998/Accepted 15 September 1998

Up to now, the only species in the complex *Borrelia burgdorferi* sensu lato known to cause Lyme borreliosis in the United States has been *B. burgdorferi* sensu stricto. However, some atypical strains closely related to the previously designated genomic group DN127 have been isolated in the United States, mostly in California. To explore the diversity of *B. burgdorferi* sensu lato group DN127, we analyzed the nucleotide sequences of the *rrf-rrl* intergenic spacer regions from 19 atypical strains (18 from California and one from New York) and 13 North American *B. burgdorferi* sensu stricto strains (6 from California). The spacer region sequences from the entire *B. burgdorferi* sensu lato complex available in data banks were used for comparison. Phylogenetic analysis of sequences shows that the main species of the *B. burgdorferi* sensu lato complex (*B. afzelii*, *B. garinii*, *B. andersonii*, *B. japonica*, *B. burgdorferi* sensu stricto, *B. valaisiana*, and *B. lusitaniae*) each form a coherent cluster. A heterogeneous group comprising strains belonging to the previously designated group DN127 clustered separately from *B. burgdorferi* sensu stricto. Within this cluster, the deep branches expressing the distances between the *rrf-rrl* sequences reflect a high level of divergence. This unexpected diversity contrasts with the monomorphism exhibited by *B. burgdorferi* sensu stricto. To clarify the taxonomic status of this highly heterogeneous group, analysis of the *rrs* sequences of selected strains chosen from deeply separated branches was performed. The results show that these strains significantly diverge at a level that is compatible with several distinct genomic groups. We conclude that the taxonomy and phylogeny of North American *B. burgdorferi* sensu lato should be reevaluated. For now, we propose that the genomic group DN127 should be referred to as a new species, *B. bissettii* sp. nov., and that other related but distinct strains, which require further characterization, be referred to as *Borrelia* spp.

In Eurasia, seven species of the complex *Borrelia burgdorferi* sensu lato have been reported. Only three of these species are associated with Lyme borreliosis. It has also been shown that each pathogenic species is associated predominantly with a given clinical presentation; *Borrelia burgdorferi* sensu stricto is associated with arthritis, *B. garinii* is associated with neuroborreliosis, and *B. afzelii* is associated with late cutaneous symptoms (2, 39). Up to now, *B. burgdorferi* sensu stricto is the only species associated with Lyme borreliosis in North America. However, two other *B. burgdorferi* sensu lato genospecies coexist in the United States, *B. andersonii* (22) and the genomic group DN127 (3, 32). *B. andersonii* seems to be restricted to a limited ecosystem involving cottontail rabbits and *Ixodes dentatus* ticks. In contrast, the genomic group DN127 appears to be involved in several enzootic transmission cycles (6, 29). A recent study demonstrated substantial genetic heterogeneity among Californian and other American strains (24). We took advantage of the unique structure of ribosomal genes in *B. burgdorferi* sensu lato to analyze the polymorphism of some strains isolated in California. A single copy of the *rrs* gene is separated by a large spacer (*rrs-rrl*; 3,000 to 5,000 bp) from two tandemly duplicated copies of *rrl* and *rrf* genes (13, 36). These two copies are separated by a small spacer, *rrf-rrl*, which is approximately 250 bp long. The genetic heterogeneity of the group DN127 was first evidenced by analysis of the restriction patterns of the *rrf-rrl* spacer (32). However, the results of DNA-DNA hybridization on a limited number of strains (32) allowed us to place them in a single genomic group. To clarify the genetic relationships between diverse North American strains, 20 atypical strains were compared with 13 *B. burgdorferi* sensu stricto strains. Identification procedures involved restriction polymorphism and sequencing studies of both the variable *rrf-rrl* spacer and the conserved *rrs* gene. Sequences of the *rrf-rrl* spacer and the *rrs* gene were used in a phylogenetic analysis. Some Californian strains are closely related to the genomic group DN127, for which we propose the name of *B. bissettii* sp. nov. Other atypical strains which do not fall into this group are designated merely as *Borrelia* spp. in this study. The latter strains cannot be assigned to specific genomic groups until more isolates representative of each group are available for further characterization.

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MATERIALS AND METHODS

Bacterial strains and DNA preparation. The designations and origins of the *Borrelia* strains used in this study are given in Table 1. The uncloned strains were grown in BSK II medium at 30°C. DNA was extracted by using the Dynabeads DNA direct kit (Dynal), a method based on DNA separation by biomagnetic beads as previously described (17). DNA samples were stored at −20°C until use for PCRs.

Analysis of restriction patterns of *rrf-rrl* spacer and sequencing. The restriction pattern analysis of amplified *rrf-rrl* spacer was performed by using primers 1 and 2 as described previously (32). *Mse*I and *Dra*I restriction patterns were used to compare the strains. The *rrf-rrl* spacer was sequenced by a solid-phase approach, using the Cy5-AutoRead sequencing kit with an ALF express automatic sequencer (Pharmacia) (17). To amplify the *rrf-rrl* spacer, we used primers A

TABLE 1. *B. burgdorferi* sensu lato isolates evaluated in this study

Strain	Accession no.		Source	Geographic location in the U.S.
	rrf-rrl spacer	*rrs* gene		
B. burgdorferi sensu stricto				
CA423	AJ006361[a]	NA[b]	*I. spinipalpis*	California
CA19 (35)[d]	AJ006360[a]	AJ224137[a]	*I. pacificus*	California
B31^T	L30127	M59293	*I. scapularis*	New York
VS2	AJ006510[a]	NA	*I. scapularis*	Shelter Island
SON328	AJ006512[a]	NA	*I. pacificus*	California
SON188	AJ006511[a]	NA	*I. pacificus*	California
SON2110	AJ006508[a]	NA	*I. pacificus*	California
MEN 115	AJ006504[a]	NA	*I. pacificus*	California
NY1-86	AJ006505[a]	NA	Human	New York
297	AJ006507[a]	X85204	Human	Connecticut
19535	AJ006503[a]	NA	*Peromyscus leucopus*	New York
26816	AJ006506[a]	NA	*Microtus pennsylvanicus*	Rhode Island
Cat flea	AJ006509[a]	NA	*Ctenocephalides felis*	Tennessee
B. bissettii sp. nov.				
CA394	AJ006359[a]	NA	*I. spinipalpis*	California
CA395	AJ006363[a]	NA	*I. spinipalpis*	California
CA370	AJ006364[a]	NA	*Neotoma fuscipes*	California
CA372	AJ006370[a]	NA	*N. fuscipes*	California
CA378	AJ006367[a]	NA	*N. fuscipes*	California
CA27 (35)	AJ006362[a]	NA	*I. pacificus*	California
DN127^T (32)	L30126	AJ224141[a]	*I. pacificus*	California
25015 (32)	L30122	AJ224138[a]	*I. scapularis*	New York
CA55 (32)	L30124	AJ224140[a]	*I. neotomae*[c]	California
CA127 (32)	NA	NA	*I. neotomae*	California
CA128 (32)	AJ006365[a]	AJ224139[a]	*I. neotomae*	California
Borrelia spp.				
CA28 (35)	AJ006375[a]	AJ224131[a]	*I. pacificus*	California
CA29 (35)	AJ006373[a]	AJ224135[a]	*I. pacificus*	California
CA8 (35)	AJ006369[a]	AJ224134[a]	*I. pacificus*	California
CA31 (35)	AJ006372[a]	AJ224132[a]	*I. pacificus*	California
CA446	AJ006366[a]	AJ224130[a]	*Dipodomys californicus*	California
CA443	AJ006368[a]	NA	*D. californicus*	California
CA404	AJ006371[a]	NA	*D. californicus*	California
CA13 (35)	AJ006374[a]	AJ224133[a]	*I. neotomae*	California
CA2 (32)	L30123	AJ224136[a]	*I. neotomae*	California

[a] Sequences determined in this study.
[b] NA, not available.
[c] Now *I. spinipalpis* (27).
[d] Numbers in parentheses indicate references.

(5′-ATTACCCGTATCTTTGGC-3′) and D (5′-TCAATAAATGTTTGCTTCT C-3′), with one of the two primers biotinylated at the 5′ end. The biotinylated strand was then immobilized through the interaction between biotin and streptavidin by using the Dynabeads M-280 streptavidin kit (Dynal) according to the manufacturer's instructions. Sequencing was performed by using the sequencing primers INS1 (5′-GAAAAGAGGAAACACCTGTT-3′) in the *rrf* gene and INS4 (5′-AGCTCCTAGGCATTCACCAT-3′) at the 5′ end of the *rrl* gene.

***rrs* sequencing.** Amplification and sequencing of the *rrs* gene were done as previously described (17).

Sequence alignments and phylogenetic analysis. Sequences were aligned both manually on VSM software V.2.0 written by B. Lafay and R. Christen (33) as described recently (10) and by using the multisequence alignment program Clustal V (10). Phylogenetic trees were constructed with distance matrix data (calculated by the method of Jukes of Cantor [14]) and both the neighbor-joining (NJ) method (34) and the unweighted pair group with mathematical average (UPGMA) (37) methods in MEGA software (15). A parsimony method in MEGA was used to analyze the *rrf-rrl* intergenic spacer sequences of select strains.

Characterization of the *rrs-rrl* spacer. The size of the *rrs-rrl* spacer was determined after amplification by using primer S15 (5′-GGGCCTTGTACACACCG CCC-3′) at the 3′ end of *rrs* and primer INS4 as given above. The PCR mixture (50 μl) contained 10 ng of DNA in 5 μl, 50 mM Tris-HCl, 1.5 mM $MgCl_2$, 20 mM NH_4SO_4, 200 μM each of the four deoxynucleoside triphosphates, 10 pmol of each primer, and 0.45 U of Hot *Tub* DNA polymerase (Amersham Life Science). The PCR was carried out for 30 cycles with an amplification profile of denaturation at 93°C for 15 s and then simultaneous annealing and extension at 60°C for 8 min, with a final extension step at 60°C for 10 min.

PCR products (10 μl) were digested with 5 U of *Hin*fI (Biolabs) in a total volume of 20 μl. Digested fragments were analyzed by electrophoresis on a 1.2% agarose gel stained with ethidium bromide.

Nucleotide sequence accession numbers. The nucleotide sequences of *rrf-rrl* spacer regions or *rrs* genes from *B. burgdorferi* sensu lato isolates have been deposited in EMBL data bank and assigned accession nos. AJ006359 to AJ006375, AJ006503 to AJ006512, and AJ224130 to AJ224141 (see Table 1).

RESULTS

Individualization of *B. bissettii* deduced from the analysis of the *rrf-rrl* spacer. Analysis of *Mse*I restriction patterns of amplification products of the spacer between the two tandem copies of the *rrl-rrf* ribosomal genes from North American strains revealed 11 different patterns (Table 2). These patterns were not identical to any of the patterns recorded previously for *B. burgdorferi* sensu lato species and genomic groups. However, they were very similar to the pattern of strains belonging to *B. burgdorferi* sensu stricto (Fig. 1). The analysis of patterns

TABLE 2. *Mse*I and *Dra*I restriction fragments of amplified *rrf-rrl* spacer

Strain(s)	Size[a] (bp) of fragments produced by:	
	*Mse*I	*Dra*I
B. burgdorferi sensu stricto		
$B31^T$	107, 52, 38, 29, 28	144, 53, 29, 28
CA19, CA423	107, 52, 40, 29, 28	146, 53, 29, 28
B. bissettii sp. nov.		
$DN127^T$, CA27, CA127, CA372, CA378, CA394	107, 52, 38, 33, 27	144, 53, 33, 27
CA128, CA395	107, 52, 38, 29, 27	144, 53, 29, 27
CA370	107, 53, 38, 28	144, 82
CA55	107, 52, 38, 29	144, 53, 29
25015	107, 52, 34, 27, 17, 12, 4	174, 53, 27
Borrelia spp.		
CA2	90, 51, 40, 28, 22, 17, 7	146, 109
CA31, CA404, CA443, CA446	107, 51, 37, 30, 28	173, 80
CA8, CA29	107, 51, 40, 28, 16, 13	146, 80, 16, 13
CA13	107, 52, 40, 17	146, 53, 17
CA28	90, 54, 38, 29, 17	144, 55, 29

[a] The exact sizes were deduced from *rrf-rrl* spacer sequences.

obtained after restriction by *Dra*I confirmed this heterogeneity (Table 2).

Some strains (CA27, CA372, CA378, and CA394) exhibited exactly the same pattern as strain DN127, the type strain of the previous genomic group DN127.

The *rrf-rrl* spacer region (32) of strains tested ranged from 216 to 257 bp. The percent identity in pairwise alignments of sequences from *Borrelia* spp. strains ranged from 85.3 to 100 (data not shown). However, all strains with identical *Mse*I or *Dra*I patterns exhibited 100% sequence identity, except strain CA404 which differed by one nucleotide from strains CA31, CA443, and CA446. To compare the polymorphism, the spacer regions from 10 American strains previously identified as *B. burgdorferi* sensu stricto were sequenced. In contrast with the extreme diversity in *Borrelia* spp., considerable homogeneity characterized the sequences of *B. burgdorferi* sensu stricto strains, as nucleotide substitutions or deletions occurred in only five positions.

The NJ and UPGMA distance methods were used to construct phylogenetic trees from sequences obtained in this study. An example of a tree drawn by the UPGMA distance method is shown in Fig. 2. Each previous species, namely, *B. garinii*, *B. afzelii*, *B. valaisiana*, *B. lusitaniae*, *B. tanukii*, *B. turdi*, *B. andersonii*, and *B. japonica*, clustered separately. One large and heterogeneous cluster of 32 sequences comprises *B. burgdorferi* sensu stricto, the strains identified as belonging to *B. bissettii* sp. nov. (formerly the genomic group DN127, DN127, CA128, CA55, and 25015), and all strains with atypical *Mse*I and *Dra*I patterns (*Borrelia* spp.). Within this cluster, all strains belonging to *B. burgdorferi* sensu stricto are closely related, which contrasts with the strains of *Borrelia* spp. that are scattered on several branches. Among the atypical strains, two strains (CA19 and CA423), despite slight differences in their *Mse*I patterns, fell into the *B. burgdorferi* sensu stricto cluster. Given the large diversity exhibited by the *Borrelia* spp., segregation of some strains did not correlate precisely with different trees drawn by phenetic (Fig. 2) or cladistic methods (data not shown). For example, the placement of strain CA2 was uncertain, as was the placement of strains CA29 and CA8, because they constituted a separate cluster comprising four distinct branches together with Californian strains CA404, CA443, CA446, and CA31 and *B. andersonii* in the tree drawn with the NJ distance method (data not shown).

However, the branch consisting of strains previously placed in the group DN127 (DN127, CA55, CA128, and 25015), and six additional strains (CA27, CA370, CA372, CA378, CA394, and CA395) was constant, irrespective of the method used to construct the trees.

New genomic groups deduced from *rrs* sequences. To provide an alternative assessment of phylogenetic relationships between such divergent strains, we sequenced the entire *rrs* gene from strains representing each of the main branches in the *rrf-rrl* trees. A phylogenetic tree showing the result of the NJ analysis of sequences is shown in Fig. 3. The assignment of strain CA19 in *B. burgdorferi* sensu stricto was confirmed. The results showed that strains of *Borrelia* spp. significantly diverge at a level compatible with distinct genomic groups. Strains CA31 and CA446 constituted one group. Strains CA8 and CA29 comprised another group. Strains CA2 and CA28 were located on two different branches according to the *rrf-rrl* sequence. They clustered together by *rrs* sequence, but their genetic distance is relatively large. The status of strain CA2 was not clear on the basis of DNA-DNA hybridization data (32). However, our present results suggest that strains CA2 and CA28 should constitute a new genomic group. Strains CA128, CA55, and 25015 segregated with strain DN127. Whether strain CA13 belong to the latter genomic group remains unknown. The clustering of strains of *Borrelia* spp. was consistent with that obtained by the UPGMA analysis (data not shown).

Polymorphism of the *rrs-rrl* spacer. It has been shown previously that the size of the spacer between the *rrs* gene and the

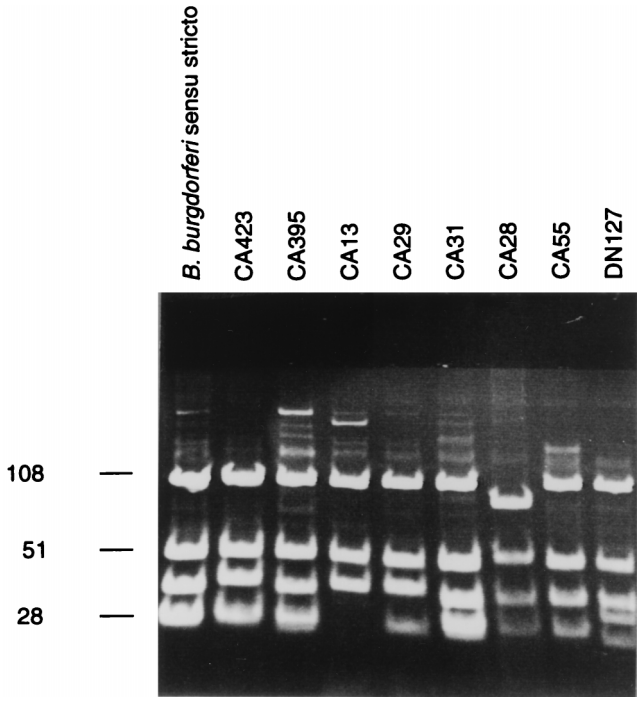

FIG. 1. *Mse*I restriction polymorphism of the amplified *rrf-rrl* spacer from Californian strains. DNA was electrophoresed on a 16% acrylamide gel, stained with ethidium bromide, and UV illuminated. The species assignment of strains is given in Table 1. The molecular sizes of DNA fragments (in base pairs) are shown to the left of the gel.

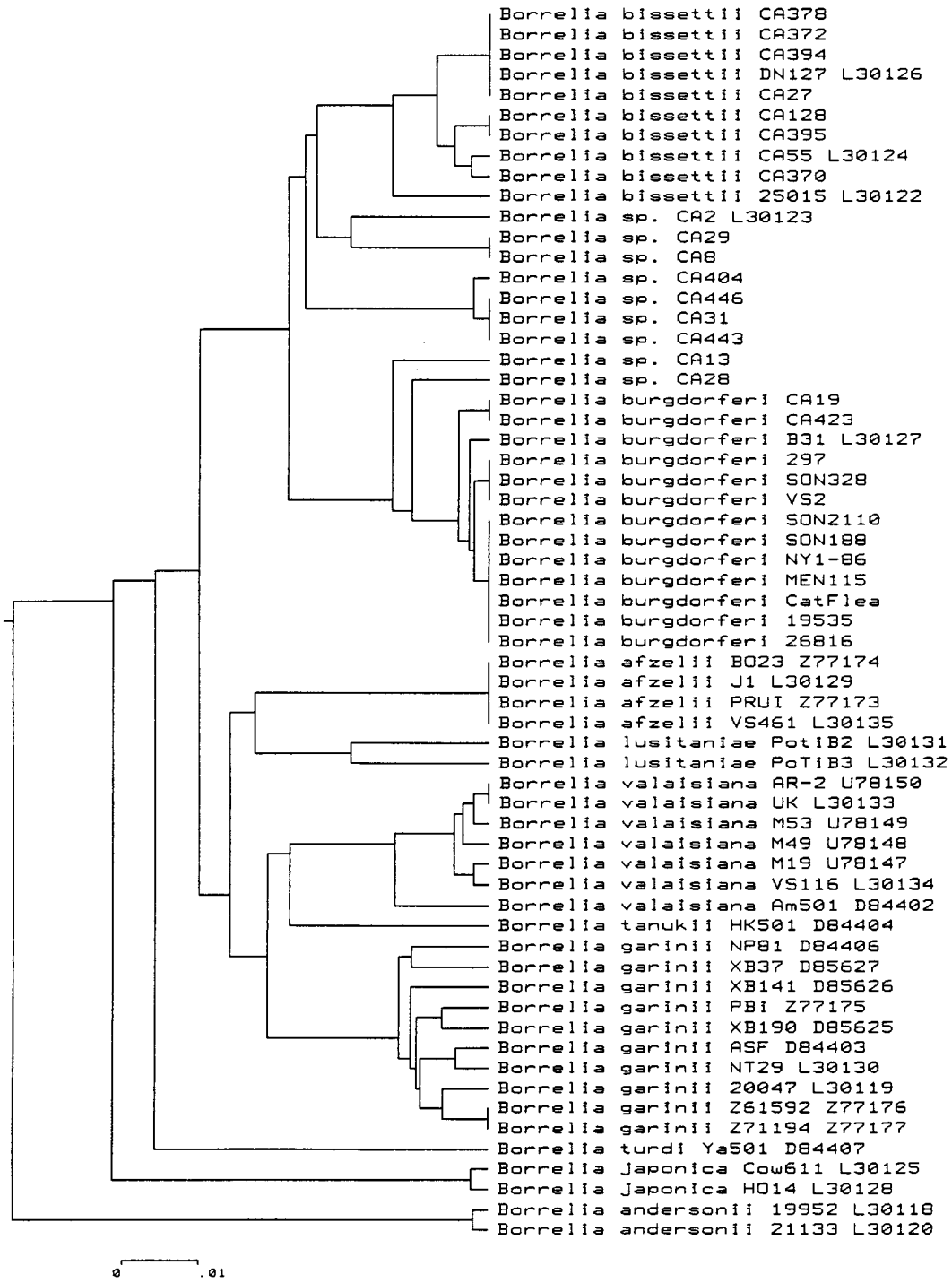

FIG. 2. Phylogenetic tree based on a comparison of the *rrf-rrl* sequences of *B. burgdorferi* sensu lato. The branching pattern was generated by the UPGMA method. The bar represents 1% divergence.

first copy of the *rrl* gene varied among different *Borrelia* species (28). The size of the *rrs-rrl* spacer allowed *Borrelia* species to be distinguished by decreasing size order from 5,000 bp for *B. afzelii* to 3,000 bp for *B. burgdorferi* sensu stricto and *B. andersonii* (Fig. 4). As shown in Fig. 4, strains evaluated in this work exhibited PCR products of two different sizes. All strains assigned to *B. bissettii* sp. nov. (DN127, CA55, 25015, CA27, CA394, CA395, CA370, CA372, and CA378), as well as four strains of *Borrelia* spp. (CA31, CA404, CA443, and CA446) exhibited a *rrs-rrl* spacer with an identical size of approximately 500 bp larger than that of *B. burgdorferi* sensu stricto. Other strains of *Borrelia* spp. had a *rrs-rrl* spacer whose size was the same as that of *B. burgdorferi* sensu stricto. The analysis of the *Hin*fI restriction pattern of the *rrs-rrl* spacer PCR product has

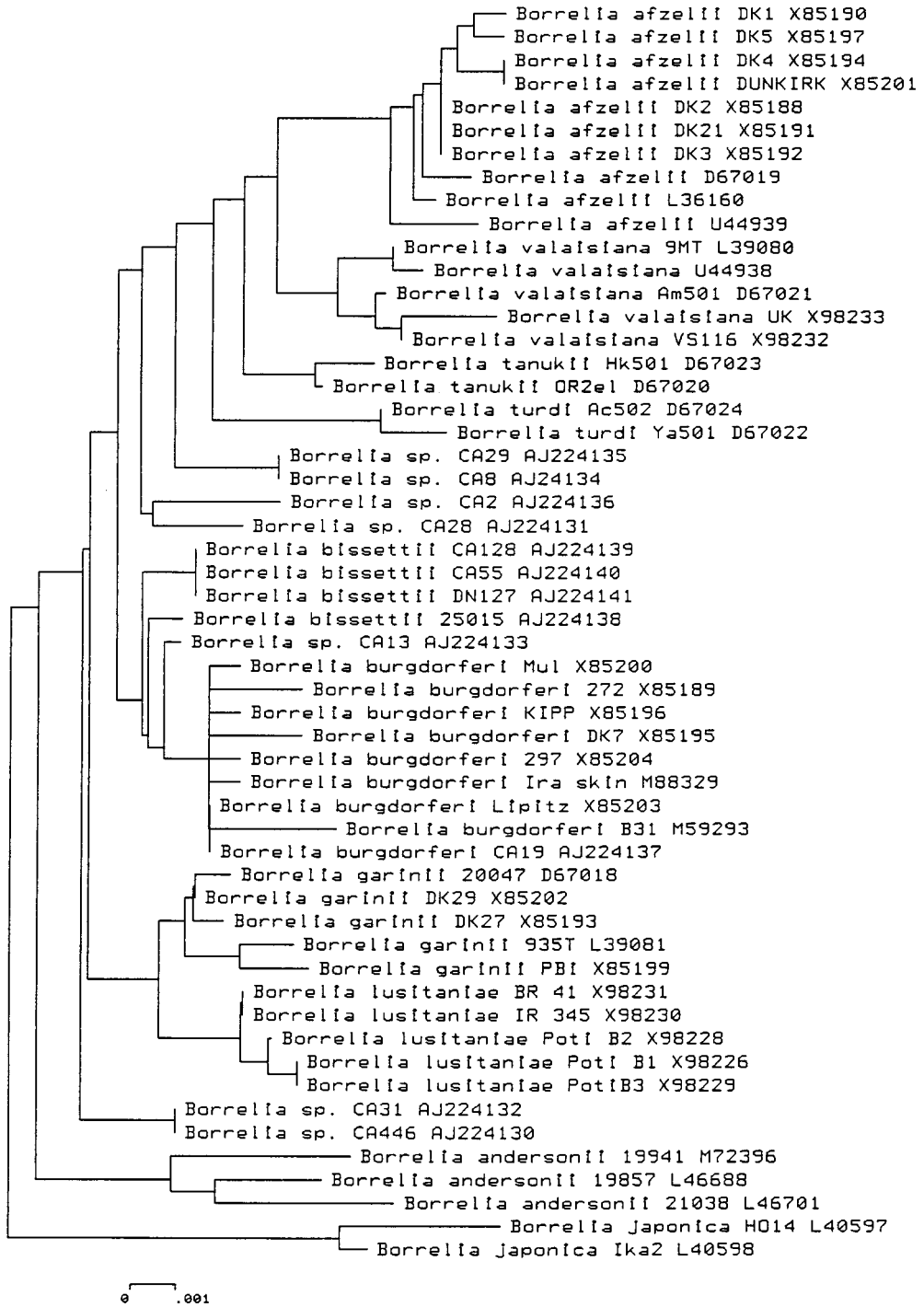

FIG. 3. Phylogenetic tree based on a comparison of the *rrs* sequences of *B. burgdorferi* sensu lato. The branching pattern was generated by the NJ method. The bar represents 0.1% divergence.

been proposed for typing of *B. burgdorferi* sensu stricto (18, 19). As described earlier (18), we also found two DNA fragment patterns among strains of *B. burgdorferi* sensu stricto. In contrast to these findings, a strong polymorphism was observed among atypical strains (Fig. 5). Ten distinctive patterns recorded from 17 atypical strains are shown in Fig. 5. Notably, the analyses of polymorphism of both the large *rrs-rrl* spacer and the small *rrf-rrl* spacer produced comparable groupings of strains.

DISCUSSION

Since the first description of *B. burgdorferi* in 1982 (7), it has been assumed that strains in the United States were more

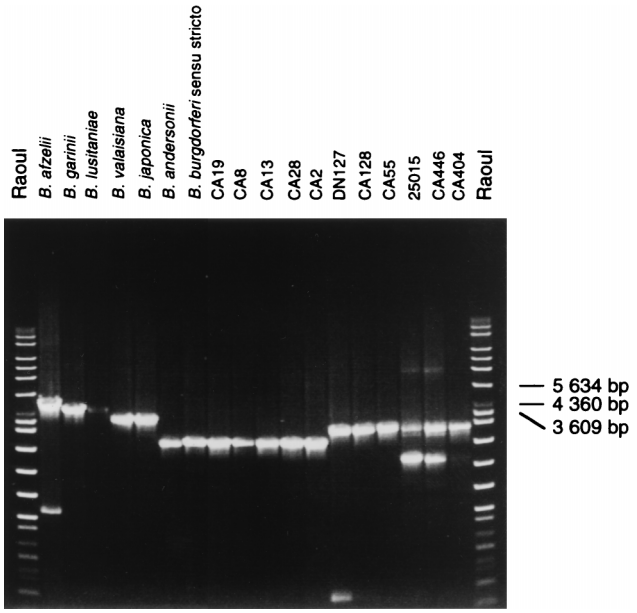

FIG. 4. PCR products of *rrs-rrl* spacer from *B. burgdorferi* sensu lato strains. The species assignment of strains is given in Table 1. Amplification was carried out by using the S15-INS4 primer set. DNAs were electrophoresed on a 0.6% agarose gel, stained with ethidium bromide, and UV illuminated. Molecular size standards Raoul (Appligene) were used.

homogeneous than the European strains (8, 9). However, an increasing number of atypical strains have been recognized in the United States, particularly in the 1990s (1, 5, 9, 12, 16, 21, 24, 35, 41, 42). Some of these strains were identified as belonging to the species *B. andersonii* (22), whereas others constituted a new genomic group called group DN127 (3, 32). Considerable phenotypic heterogeneity was found among strains described from California and Colorado (26, 35), and substantial genetic diversity was reported among a large number of North American strains (24) on the basis of genomic macrorestriction analysis and *ospA* and *rrl* gene sequencing. Our study emphasizes that the genetic diversity among American strains is much greater than previously thought.

Phylogenetic analyses of *rrs* gene sequences have often been used to evaluate the taxonomic relatedness of *B. burgdorferi* sensu lato strains (11, 17, 20, 40). The results of these analyses correlated well with data from DNA-DNA hybridizations (32). The *rrs* gene sequence analysis of atypical strains confirmed the foregoing results and revealed at least four groups which appear to represent heretofore undescribed genospecies. CA19 belongs to *B. burgdorferi* sensu stricto. Three new genomic groups were CA29-CA8, CA2-CA28, and CA31-CA446, respectively. The taxonomic position of strain CA13 remains unclear. A polymorphism was observed in the *rrf-rrl* restriction patterns of the strains within the genomic group DN127. Also, some differences were previously reported for the physical maps of strains DN127 and CA55, which were classified in two separate groups (8). However, data originated from the single gene locus *hbb* (38), as well as information acquired by multilocus enzyme electrophoresis, involving the whole genome (4) were consistent with data from DNA-DNA hybridizations showing that DN127 and CA55 do belong to the same species (32). Moreover, the *rrs* sequences of these two strains are 100% identical. To clarify the taxonomic status of *B. burgdorferi* sensu lato in the United States, we propose the name *B. bissettii* sp. nov. for the genomic group DN127 in honor of Marjorie L. Bissett, who with her coworker Warren Hill, first described a member of this group in 1987 (5). We refrain from naming the other genomic groups until hybridization data are available for genetic characterization. Instead, we lump them here as *Borrelia* spp. The noncoding region between the two copies of *rrf* and *rrl* genes was shown previously to reflect the taxonomic status of strains (32). The phylogenetic study of this region showed that strains from each *B. burgdorferi* sensu lato species clustered as expected taxonomically, and each cluster clearly diverged from others. In contrast with the high conservation of sequences within the *B. burgdorferi* sensu stricto cluster, *rrf-rrl* sequences from *Borrelia* strains exhibited an unexpected broad diversity. This region is not constrained genetically, so the number of mutational events found should reflect the relative antiquity of the members of this bacterial complex. Californian strains were found scattered in different clusters. Within each cluster, the deep branches expressing the distances between the *rrf-rrl* sequences reflect a high divergence level. According to previous studies (23, 32), these results strongly suggest that these strains should constitute new genomic groups. Despite some discrepancies between the different trees, all are consistent with the placement of strains belonging to *B. bissettii* sp. nov. in a distinct cluster. Within this cluster, only strain 25015 was located on a separate branch.

In opposition with what is usually thought, more than two *B. burgdorferi* sensu lato species (*B. burgdorferi* sensu stricto and

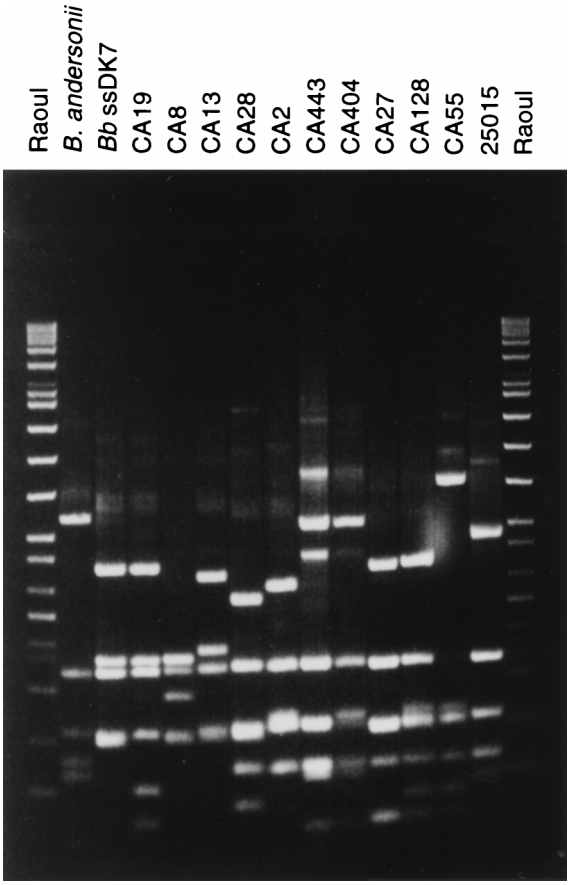

FIG. 5. Restriction patterns of Californian strains. The species assignment of strains is indicated in Table 1. DNAs from amplified *rrs-rrl* spacers were digested by *Hin*fI. DNAs were electrophoresed on a 1.2% agarose gel, stained with ethidium bromide, and UV illuminated. Molecular size standards Raoul (Appligene) were used.

B. andersonii) seem to occur in the United States. *B. burgdorferi* sensu stricto is transmitted primarily by *Ixodes scapularis* and *Ixodes pacificus* ticks, whereas the new genomic groups described here are associated with these two ticks plus *Ixodes neotomae* (now *Ixodes spinipalpis* [27]) and some of its rodent hosts. Moreover, *I. spinipalpis* also can harbor *Borrelia* from other genomic groups, since strains CA2 and CA13 represent two distinct groups. Thus, there are a variety of ticks and reservoir host for *B. burgdorferi* sensu lato in the United States. It is not known whether *B. bissettii* sp. nov. and the other novel genomic groups can infect humans. All the strains used in this study were isolated from ticks or small mammals. As stated by Oliver (29), clinical manifestations of Lyme borreliosis in the southern United States are mild and some cases may be asymptomatic. Thus, the roles of *B. bissettii* sp. nov. and *Borrelia* spp. in producing Lyme borreliosis remain to be demonstrated. In addition, Picken et al. (30, 31) recently described human strains from Slovenia related to strain 25015, which belongs to *B. bissettii* sp. nov. on the basis of large restriction fragment pattern, protein, and plasmid profile analyses. If the strains from Slovenia are true members of *B. bissettii* sp. nov., the pathogenicity of this species should be evaluated there as well as in the United States. In Europe, three species are known to be pathogenic for humans; in the United States, all strains isolated from humans so far belong to *B. burgdorferi* sensu stricto. This could mean that *I. spinipalpis* does not transmit *Borrelia* to humans or that these new genomic groups are nonpathogenic for humans. This latter hypothesis seems more likely, since *I. pacificus* and *I. scapularis* frequently bite humans and occasionally harbor such strains. Moreover, *I. spinipalpis* primarily infests rodents and lagomorphs and rarely attaches to humans (25). However, despite the great number of strains isolated from *I. scapularis* in the United States, 25015 is the only strain recovered from this tick that belongs to a species other than *B. burgdorferi* sensu stricto. Also, this strain is genetically distant from other strains within *B. bissettii* sp. nov. This fact could reflect genetic adaptation to an unusual vector. The heterogeneity encountered among *B. burgdorferi* sensu lato in the United States might be compared to that described in Europe and Asia. On the latter two continents, *B. garinii* comprises a more heterogenous collection than *B. afzelii* and *B. burgdorferi* sensu stricto. Aside from the three pathogenic species, nonpathogenic species, such as *B. valaisiana*, *B. lusitaniae*, or *B. japonica*, coexist. More data are needed to understand the significance of the diversity of *B. burgdorferi* sensu lato and their role in human Lyme borreliosis.

ACKNOWLEDGMENTS

We thank T. G. Schwan and R. N. Brown for supplying some of the strains, I. Saint Girons for critical reading of the manuscript, and E. Bellenger for technical assistance.

We thank the Pasteur Institute for supporting this work. Also, many of the Californian strains were acquired during ecologic studies supported in part by funding to R.S.L. from the U.S. National Institutes of Health (grant AI22501) and the Centers for Disease Control and Prevention (cooperative agreement U50/CCU906594).

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